

External validation of a tumor derived 5-gene prognostic signature for recurrence (R) of stages I/II colon and stage I rectal cancer following potentially curative resection

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ABSTRACT

Background: Optimizing post-operative clinical management for early-stage (I/II) colorectal cancer (CRC) patients (pts) is a significant unmet medical need. We hypothesized that a molecular prognostic test using primary CRC tissue would better predict the chances of tumor R within 36 months (mo) than current NCCN Clinical Practice Guidelines (NCCN).

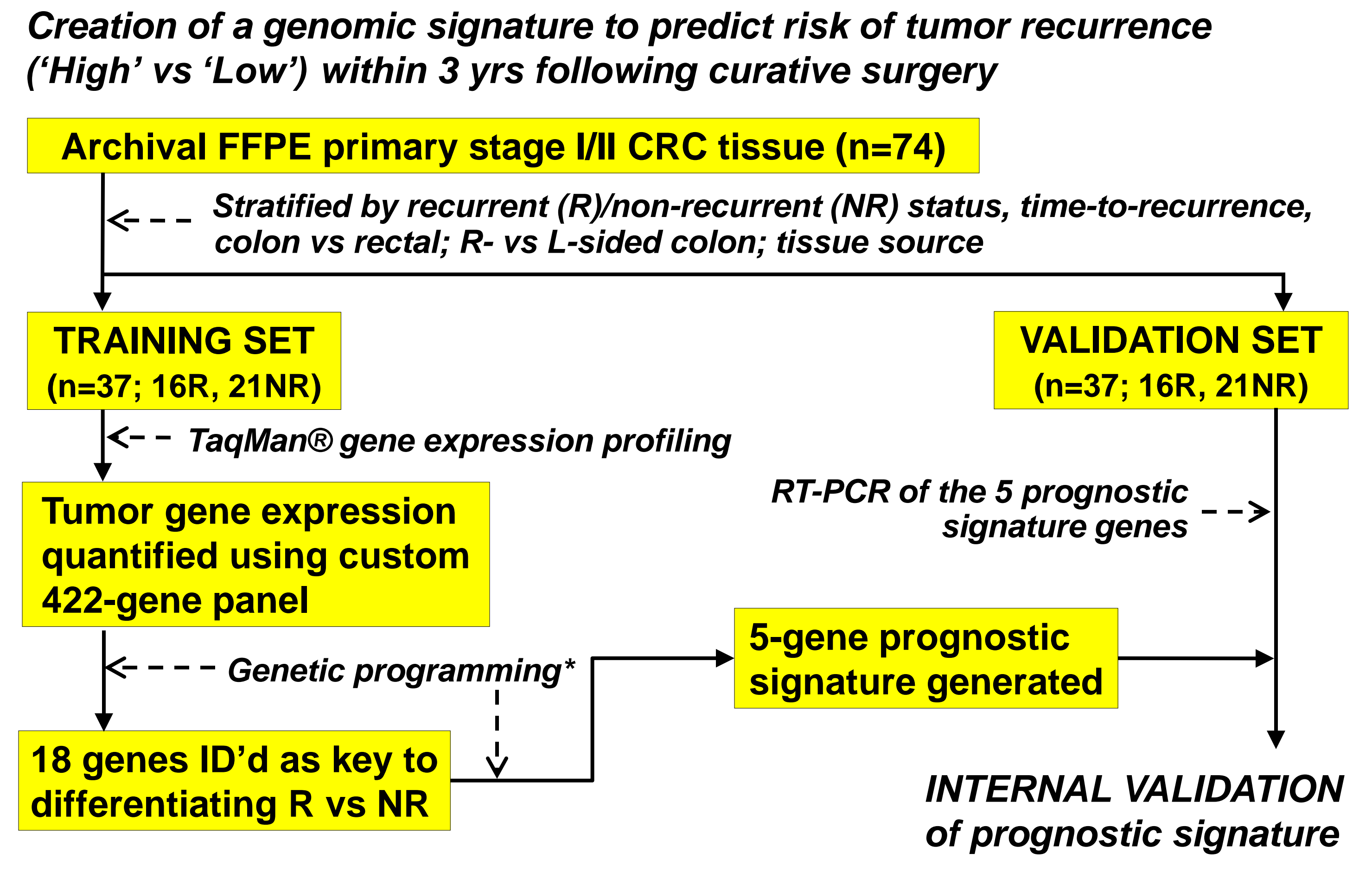
Methods: Pts had tumor R by 36 mo (n=46) or confirmed non-recurrence (NR) for ≥36 mo (n=69) after surgery; none had received neoadjuvant or adjuvant therapy. Archival formalin-fixed paraffin-embedded primary adenocarcinoma tissues (median storage 7 years; range 4-15) obtained at initial surgical resection with curative intent were retrieved for 86 stage I/II (pT1-4 pN0 M0) colon cancer and 29 stage I (pT1-2, pN0 M0) rectal cancer pts from 2 US and 2 European sites that were different from those previously used to generate the molecular test. Tumor gene expression was assessed by RT-PCR with custom 384-well TaqMan® Low Density Arrays (Applied Biosystems) using RNA that had satisfied a set of rigorous quality control parameters.

Results: For stages I/II CRC (n=115), the dichotomous rule correctly classified 32/46 R and 38/69 NR pts: sensitivity (S) 0.70, specificity (SP) 0.55. 'High risk' pts had a significantly higher probability of R by 36 mo than 'low risk' pts: PPV 0.51, NPV 0.73; hazard ratio (HR) 2.06 (95% CI: 1.10 to 3.86; p=0.020). NCCN (V.1.2011) was not able to differentiate 36-mo R vs NR in this population: S 0.72, SP 0.42, PPV 0.45, NPV 0.69; HR 1.38 (95% CI: 0.73 to 2.63; p=0.315). The SP of the molecular test was significantly greater than that for NCCN (p=0.05). For stage I pts (n=29; 13R, 16NR), the prognostic accuracy of the test (0.79; 23/29) surpassed that for NCCN (0.55; 16/29).

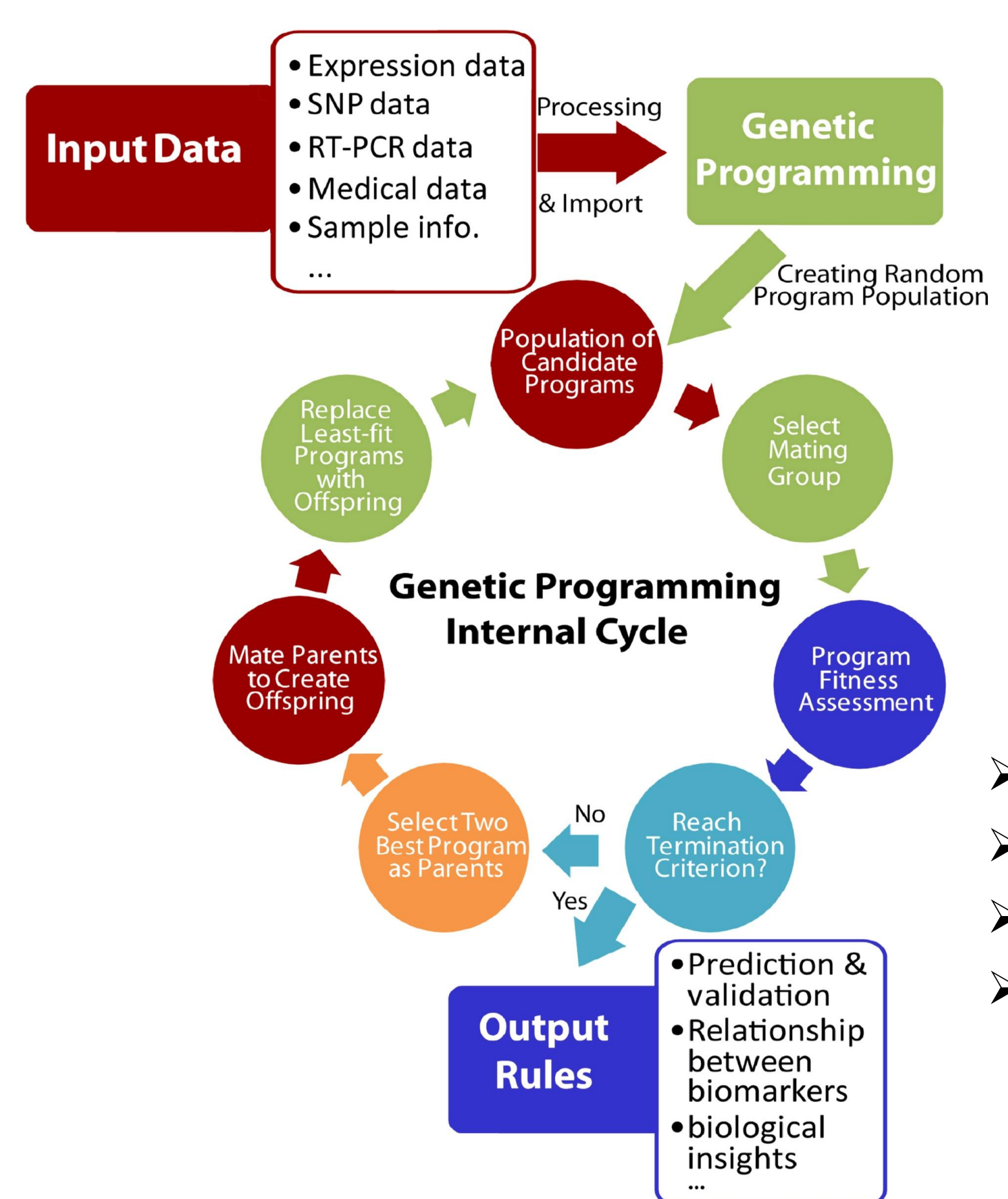
Conclusions: External validation of a 5-gene prognostic rule confirmed its ability to differentiate early stage CRC pts at high risk vs low risk for R within 36 mo after surgery better than current NCCN Guidelines, especially for stage I. The improved specificity and high sensitivity of the molecular test confirm its potential utility for optimizing post-op clinical management of early stage CRC.

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METHODS & DESIGN



***GENETIC PROGRAMMING (Ref 1):**



- A machine-learning analytic technique
- Can integrate large/diverse data sets
- Unbiased search for key variables
- Creates concise, human readable predictive rules

PATIENT CHARACTERISTICS – External Validation Set

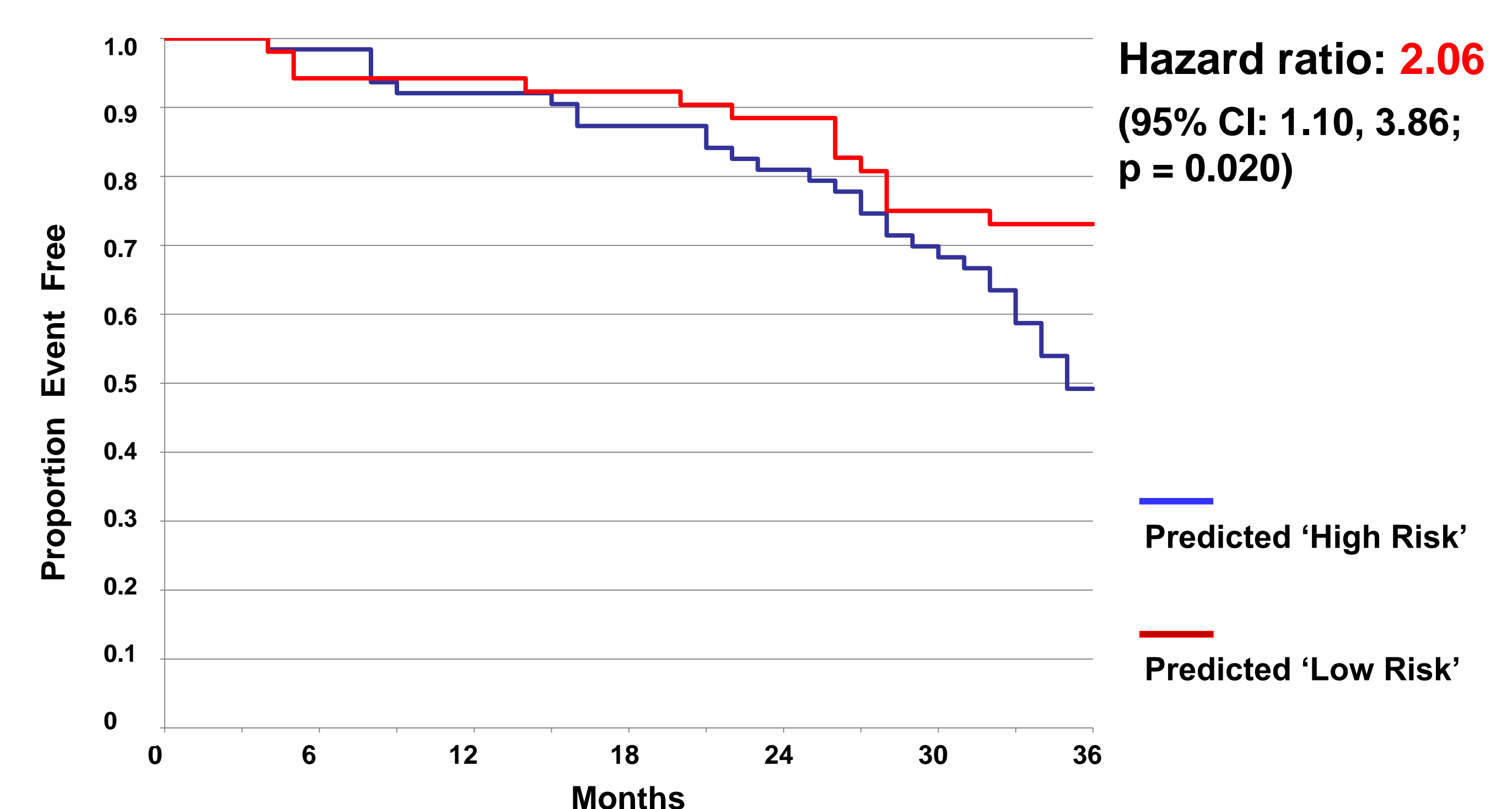
ALL PATIENTS (n=115)	Recurrent (n=46)	Non-recurrent (n=69)
Age at surgery, median yrs (range)	63 (48-79)	65 (36-90)
Male/Female, # pts	15/31	41/28
T-stage (T1/T2/T3/T4), # pts	1/12/14/19	3/13/31/22
Colon ca stages I/II, # pts	8/33	15/53
• R-sided / L-sided, # pts	23/18	42/26
Rectal ca stage I, # pts	5	1
# LN's examined, median (range)	3 (2-15)	6 (2-59)
Grade I/II/III, # pts	6/36/4	12/53/4

RESULTS

❖ PROGNOSTIC SIGNATURE

External Validation Set (n=115; 46R, 69NR)	CRC outcome at 36 mo		Sensitivity 32/46 = 0.70 Specificity 38/69 = 0.55 PPV 32/63 = 0.51 NPV 38/52 = 0.73 Accuracy [32+38]/115 = 0.61
	Recur	Not Recur	
Predicted Risk of CRC recurrence within 36 mo			
'High'	32	31	
'Low'	14	38	

Recurrence-free survival curve using ext validation set:



❖ NCCN CLINICAL PRACTICE GUIDELINES IN ONCOLOGY (Ref 2)

External Validation Set (n=115; 46R, 69NR)	CRC outcome at 36 mo		'High risk' for recurrence: All stage II rectal ca; stage II colon ca with ≥1 of these features: <12 LN's examined, T4, grade 3-4, lymphatic/vascular invasion, bowel obstruction, localized perforation, or close, indeterminate or (+) margins
	Recur	Not Recur	
Predicted Risk of CRC recurrence within 36 mo			
'High'	33	40	
'Low'	13	29	

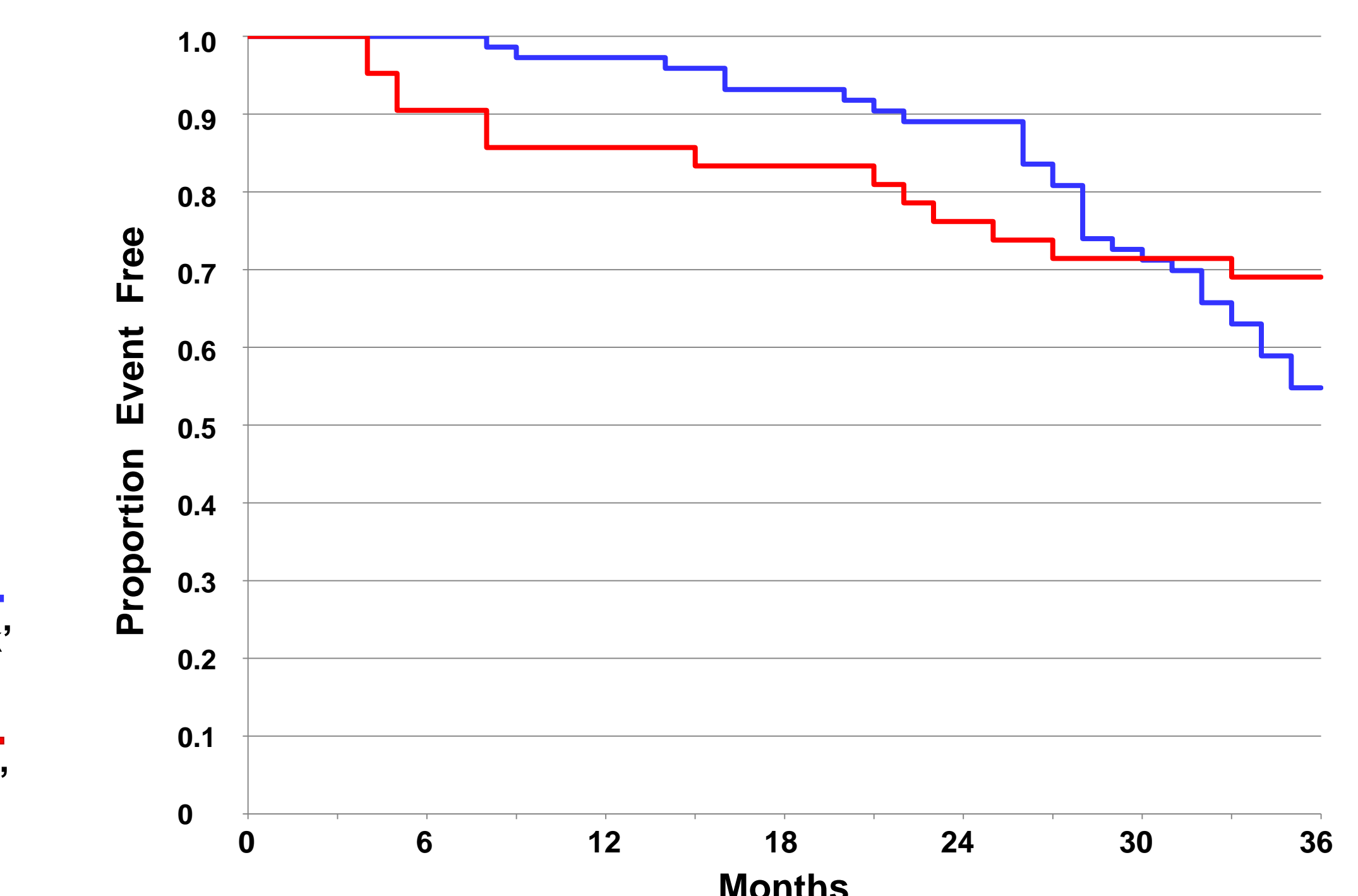
Sensitivity 24/33 = **0.72**
 Specificity 15/39 = **0.42**
 PPV 24/48 = **0.45**
 NPV 15/24 = **0.69**
 Accuracy [33+29]/115 = **0.54**

Hazard ratio: 1.38

(95% CI: 0.73, 2.63; p = 0.315)

Predicted 'High Risk' (blue line)
 Predicted 'Low Risk' (red line)

Recurrence-free survival curve using ext validation set:



CONCLUSIONS

- 1) Genetic programming (GP) analysis of gene expression profiles from formalin-fixed paraffin-embedded (FFPE) tumor tissue archived for up to 15 years can identify key genes for differentiating pts with early stage CRC that will recur vs not recur within 3 yrs after potentially curative surgery.
- 2) A GP derived 5-gene prognostic signature using FFPE tumor tissue can differentiate stage I/II colon and stage I rectal cancer pts at **high versus low risk** for recurrence within 3 yrs better than current NCCN Practice Guidelines.
- 3) The performance characteristics during external validation of this first ever combined stage I/II molecular prognostic test for CRC confirm its potential utility for optimizing post-op clinical management of early stage disease.

REFERENCES

- 1) Mitra AP, et al. The use of genetic programming in the analysis of quantitative gene expression profiles for identification of nodal status in bladder cancer. *BMC Cancer* 2006; 6:159
- 2) The NCCN Clinical Practice Guidelines in Oncology™ COLON CANCER (Version 2.2011) and RECTAL CANCER (Version 2.2011). © 2011 National Comprehensive Cancer Network, Inc. Available at: <http://www.nccn.org>